ON CARBOHYDRATE CONSUMPTION BY AZOTOBACTER CHROOCOCCUM

E. R. ALLEN

Formerly Associate in Biochemistry, Washington University School of Medicine and Visiting Investigator, Missouri Botanical Garden

In a previous paper (Allen, '19) devoted mainly to the study of the cause of beneficial action resulting from mechanical agitation of *Azotobacter* solution cultures, the defects of existing experimental methods for the study of the physiology of this organism were pointed out. The present report covers a continuance of these studies aimed at the following improvements: (1) renewal of the energy source in the cultures; (2) simultaneous determinations of nitrogen and of residual carbohydrate at short intervals; and (3) mechanical improvements.

The object of the first of these improvements was, of course, to produce heavier growths in the cultures and therefore more marked changes in the amounts of metabolic products, thus increasing the reliability of the measurements of such changes. In view of the theoretical considerations of Duclaux ('98–'00) in regard to the rate of increase in the number of cells, we were justified in expecting a mounting rate of total physiological activity as growth proceeded, until the normal rate of development was checked by the accumulation of unfavorable by-products. The method of renewal of energy-supplying material has been used, for example, with marked success by Bonazzi ('19) in his investigations of the organisms of nitrification.

The second improvement was designed to furnish a more complete picture of growth processes of *Azotobacter* than is obtainable by the determination of one, or even two, metabolic products at the end of an arbitrarily chosen incubation period. Unfortunately, the "micro" determination of carbohydrate and nitrogen on the same sample proved more difficult than was expected, and had to be postponed for the time being. The present work, therefore, lacks this improvement over that reported previously.

The third improvement was to facilitate experimental manipulation and possibly obtain even better results from mechanical agitation. The type of agitation decided upon was a slow

rate of revolution of round-bottomed culture flasks held in a horizontal position. A rotating device was constructed, which held a vertical wooden wheel, to the rim of which were attached, horizontally, four one-liter round-bottomed flasks. The wheel revolving at the rate of one revolution in five minutes kept the culture medium in a slow, uniform motion.

Only one experiment was conducted, and, as pointed out above, it was lacking in the second improvement. Carbohydrate only was determined at short intervals, and the only renewal of this material was in two of the flasks on the rotator. The results obtained were so consistently contrary to expectation that the data from even the one experiment is of some interest; that is, as measured by carbohydrate consumption, the cultures showed a declining instead of an increasing metabolic activity.

The *Azotobacter* culture used was a later generation of the same strain used in previous work. The nutrient solution was prepared as follows:

Solution A:	
Dipotassium phosphate	0.2 gm.
Sodium chloride	0.2 gm.
Dextrose	20.0 gms.
Water, distilled	500.0 cc.
Solution B:	
Magnesium sulphate	0.2 gm.
Calcium sulphate	0.1 gm.
Water	500.0 cc.
Ferric chloride (10 per cent sol.)	3 drops

Solutions *A* and *B* were mixed, and 100-cc. portions then placed in each of four one-liter round-bottomed flasks and in each of two two-liter Erlenmeyers, all flasks receiving in addition 1 gm. calcium carbonate. The flasks were then plugged with cotton and capped with beakers in the usual way. After sterilization by the intermittent method, all except one round-bottomed flask were inoculated with a spiral of a suspension prepared from an agar slant, the round-bottomed flasks placed in position on the rotator, and the Erlenmeyers on a shelf near by. The whole experiment was set up in a warm room kept at 28–30° C. by a thermostat.

The experiment was started March 14, 1919. Two days later a distinct turbidity appeared in the inoculated flasks on the

rotator, and a lesser one in the Erlenmeyers on the shelf. At short intervals 5-cc. samples were removed aseptically and submitted to duplicate sugar determinations by the modified Shaffer method described in the previous paper (Allen, '19). Cultures II and IV received on March 24, 25 cc. of an 8 per cent dextrose solution. Culture No. III was unfortunately lost at this point.

The data are reported as dextrose remaining per 100 cc. of culture solution, no correction being made for evaporation; that is, if a 5-cc. sample is found to contain 35 mgs., the value reported is 0.7000 gm., regardless of the total volume of the culture at the time.

The results appear in tabular form in tables I and II, and in graphical form in fig. 1.

TABLE I

NUMBER GRAMS RESIDUAL DEXTROSE PER 100 CC. CULTURE SOLUTION AT DIFFERENT PERIODS

Culture no.	March 17	March 19	March 24	March 28	April 2
Rotator: (round-bottomed flasks)					
I (Check)	2.1565				
II	1.2732	0.7600	0.110–2.060	1.976	1.255
III	1.4822	1.3507	0.908–Lost		
IV	0.9410	0.5700	0.069–2.023	1.842	1.358
Shelf: (Erlenmeyer flasks)					
V	1.7257		1.636	1.674	1.523
VI	1.5962		1.418	1.507	1.389

TABLE II

MILLIGRAMS DEXTROSE CONSUMED PER DAY IN EACH PERIOD

Culture no.	First 3 days	Next 2 days	Next 5 days	Additional 4 days	Additional 5 days
I Check					
II	295	256	130	21	144
III	225	65	88		
IV	405	185	100	45	97

In constructing the graphs the curves were plotted in the normal manner, with the amounts of dextrose on the Y axis and time on the X axis for the first ten days of the incubation period. In order to make the curves for cultures II and IV continuous and harmonic, the Y ordinate is shifted for these

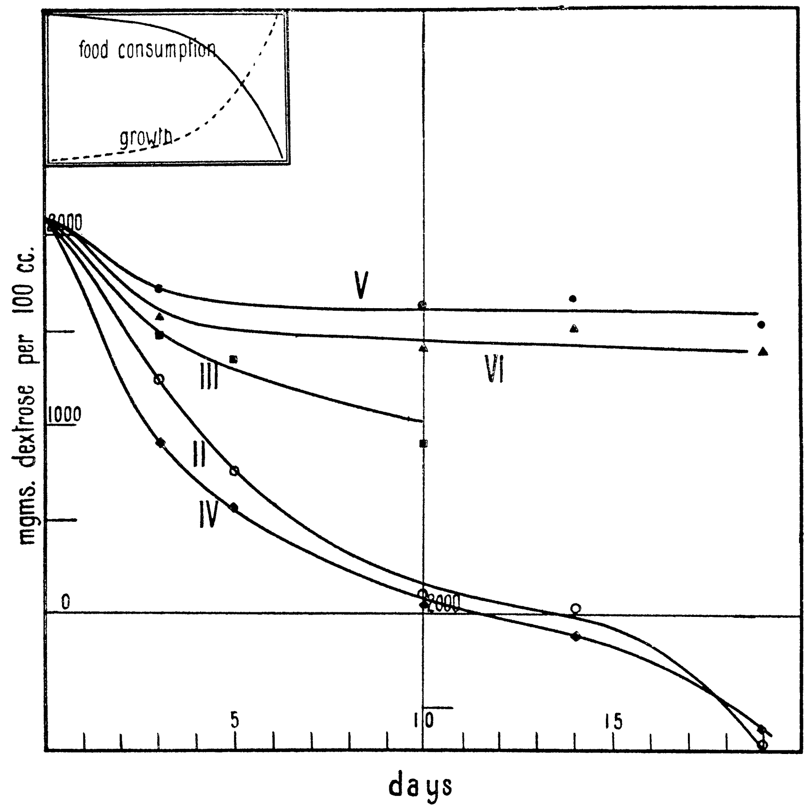

FIG. 1

cultures at the end of 10 days so that the 2000-mgm. point is on the original X axis. The dotted curve in the insert is the Duclaux curve for increase in number of cells, while the solid line represents the type of curve predicted on the assumption that carbohydrate consumption is a simple function of cell multiplication.

The results show that the rate of carbohydrate consumption in *Azotobacter* cultures does not proceed in a manner similar to

the rate of increase in cell numbers predicted by Duclaux for bacterial cultures in general. Indeed, the curves resemble the antipode of the Duclaux curve.

It might be argued that the decrease in rate of sugar consumption with increase of time in the cultures is due to accumulation of by-products, and that this decreased activity corresponds to the falling away from the purely mathematical curve in older cultures, as discussed by Duclaux. The production of toxic substances within seven days in cultures as slow growing and as dilute as these seems to us to be unlikely. Moreover, the fact that the cultures are able to utilize a second portion of sugar seems to us to be opposed to the idea of growth-inhibiting substances in the medium.

The results show also that the cultivation of the organism under the influence of mechanical agitation of a certain type influences to a marked extent the carbon assimilation in its time relations.

On the whole, the experiment emphasizes the need for more studies on the periodic changes in the culture of this organism.

In conclusion, the writer wishes to express his thanks to Professor P. A. Shaffer for rendering this work possible; to Professor B. M. Duggar for suggesting the method of attack, and to Mr. A. Bonazzi for suggestions in regard to the arrangement of the manuscript.

Literature

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